

In the Claims

1. A method for detecting a target nucleic acid in a sample, said method comprises the steps of:

5 (a) providing an amplification reaction mixture that comprises said sample, a DNA binding agent, wherein said agent is characterized as providing a detectable signal when bound to double-stranded nucleic acid which signal is distinguishable from the signal provided by said agent when it is unbound, and reagents for amplification;

(b) determining the amount of said signal produced by the mixture of step (a);

10 (c) treating said mixture under conditions for amplifying said target nucleic acid;

(d) determining the amount of said signal produced by said mixture of step (c);

and

(e) determining if amplification has occurred.

15 2. The method of Claim 1, wherein said DNA binding agent is an intercalating agent.

20 3. The method of Claim 2, wherein said DNA binding agent is further characterized as providing an amount of detectable signal when said agent is bound to double-stranded nucleic acids that is greater than the amount of said detectable signal produced when said agent is unbound.

25 ³4. The method of Claim 2, wherein said intercalating agent is a fluorescent dye.

⁴5. The method of Claim ³4, wherein at step (e) an increase in fluorescence indicates that amplification has occurred.

30 ⁶6. The method of Claim ⁴5, wherein at steps (b) and (d) the amount of signal produced is determined by exposing said mixture to UV light, and at step (e) comparing the relative amount of signal produced at steps (b) and (d) to determine if amplification has occurred.

35 ⁶7. The method of Claim ⁴5, wherein said fluorescent dye is ethidium bromide.

⁷8. The method of Claim ⁴5, wherein the amount of signal produced is determined using a spectra fluorometer.

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~~9.~~ The method of Claim ⁴~~5~~, wherein said target nucleic acid is indicative of a genetic or infectious disease.

Sub 21
5 10. The method of Claim 5, wherein the amount of target DNA in said sample, prior to amplification, is quantitated by determining the increase in fluorescence before and after PCR.

10 11. A method for monitoring the increase in double-stranded DNA during amplification of a target nucleic acid in a sample, said method comprises the steps of:
 (a) providing a mixture that comprises a PCR containing said sample and a DNA binding agent, wherein said agent is characterized as providing a detectable signal when bound to double-stranded nucleic acid which signal is distinguishable from the signal provided by said agent when it is unbound;
15 (b) determining the amount of said signal produced by the mixture of step (a);
 (c) treating said mixture under conditions for amplifying said target nucleic acid; and
 (d) determining the amount of said signal produced by said mixture during said treating step (c).

20 ¹¹~~12.~~ The method of Claim ¹⁰~~11~~, wherein at steps (b) and (d), an optic fiber and spectra fluorometer are used to determine the amount of signal produced during said treating step.

25 ¹²~~13.~~ The method of Claim ¹¹~~12~~, wherein said DNA binding agent is an intercalating agent.

✓ ¹³~~14.~~ The method of Claim ¹¹~~12~~, wherein at step (d) the amount of signal is determined continuously throughout ^{the} amplification reaction.

30 ¹⁴~~15.~~ The method of Claim ¹²~~13~~, wherein said intercalating agent is a fluorescent dye.

35 ¹⁵~~16.~~ The method of Claim ¹⁴~~15~~, wherein said fluorescent dye is ethidium bromide.

Sub 3
~~17. A kit for amplifying a target nucleic acid, that comprises a PCR buffer that comprises and an intercalating agent.~~
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18. The kit of Claim ¹⁶~~17~~, wherein said intercalating agent is a fluorescent dye.
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19. The kit of Claim ¹⁷~~18~~, wherein said fluorescent dye is ethidium bromide.
- 5 ¹⁹
20. The kit of Claim ¹⁸~~19~~, wherein said ethidium bromide is present at a concentration suitable to provide between 0.15 μ M and 40.6 μ M dye in a PCR reaction.
- 10 ²⁰
21. The kit of Claim ¹⁹~~20~~, wherein said buffer also comprises Tris-HCl, pH 8.0-8.3 and KCl, each present in a concentration suitable for amplifying a target nucleic acid in a PCR.
- 15 ²¹
22. The kit of Claim ¹⁹~~20~~ that also comprises a DNA polymerase, $MgCl_2$, and dNTPs.

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